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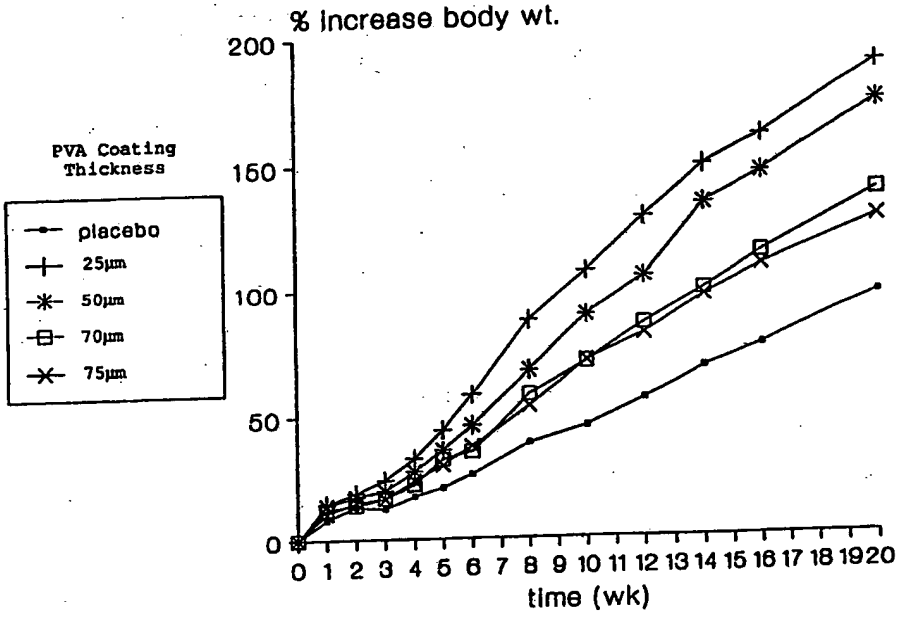

(57) Abstract

A single administration of a novel controlled release dosage form comprising a growth hormone coated with a non-covalently crosslinked water swellable polyvinyl alcohol enables the accelerated growth of non-human animal species, particularly commercially exploited teleosts, over a prolonged period, for example, longer than 20 weeks.

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"POLYVINYL ALCOHOL COATED PELLET OF GROWTH HORMONE"

FIELD OF THE INVENTION

The present invention relates to growth hormones, their formulation, and use. Generally, this invention concerns the use of growth hormones in controlled release formulations and the administration of such formulations to non-human animal species. Preferably, the present invention relates to the use of growth hormones in controlled release formulations designed to promote prolonged accelerated growth rates in commercially exploited teleosts.

BACKGROUND OF THE INVENTION

It has long been known that various growth hormones of the growth hormone-prolactin family, isolated previously from an exogenous source and then administered to an animal of interest, can promote accelerated growth rates, and in some instances, result in the production of animals larger than those otherwise occurring naturally. In addition, exogenous growth hormone administration is known to promote the production of animals with a lower body fat content than is otherwise attainable through the use of selective breeding and/or nutritionally modified diets. Thus, the use of various growth hormones in the cultivation of numerous commercially exploited non-human animal species holds out the possibility of producing larger, leaner animals more rapidly and economically.

Growth hormones traditionally have been obtained from natural animal sources, typically from

the isolated pituitary glands of freshly slaughtered animals. Because growth hormones are produced naturally in extremely small amounts and repeated growth hormone administrations over extended periods of time have been required to produce statistically significant growth rate enhancement, the isolation and purification of sufficient quantities of growth hormone from natural sources for wide spread commercial application is not economically practical. Recently, the advent of recombinant DNA technology has enabled the large-scale production of various growth hormones via the recombinant expression of genes encoding such proteins in both eucaryotic and procaryotic host cells. Therefore, the problem of producing the quantities and quality of growth hormone necessary for commercial agricultural application has now been solved. However, other problems, like protein stability, methods of administration, formulation, etc. remain and must be overcome before growth hormones produced by recombinant techniques can be applied on an industrial scale in animal husbandry.

Growth hormones are known to be relatively unstable proteins when exposed over prolonged periods to physiological temperatures similar to those encountered in warm blooded animal species. Because of this, growth hormone must currently be administered on a frequent basis to animals receiving growth hormone treatment. Attempts to incorporate growth hormone into a dosage form capable of sustaining controlled release of biologically active protein over a period of more than two weeks have, for the most part, not been successful. However, the present invention enables the production of a growth hormone formulation capable of directing the controlled release of a growth hormone to a non-human animal species over a prolonged period.

This invention has particular relevance to the commercial fish industry, although it is also applicable to a wide variety of animal species.

Worldwide, the commercial harvest of natural fish stocks has plateaued at an annual level of approximately 70 million metric tons [ICLARM (1982), Int. Center for Living Aquatic Resources Management Progress report]. As a result of the apparent maximal exploitation of wild teleost sources, coupled with a continuing growth in demand for fish products, efforts at the large-scale aquaculture of desirable fish species have been increasing. It is generally recognized that several requirements must be satisfied if commercial teleost aquaculture is to be optimized. Initially, the ability to produce gametes from broodstock and rear the resultant offspring through early life stages must be accomplished. Secondly, nutritionally adequate diets must be designed and the fish reared to an acceptable market size. Finally, the health of the cultured fish must be maintained throughout the production cycle (the time required to raise a fish from a fertilized egg to a marketable size). In addition, the commercial feasibility of large-scale aquaculture is influenced both by the length of the production cycle, which can be several years for large Pacific or Atlantic salmon or trout, and by feed costs, which can account for up to 50% of the cost of an aquaculture operation [Gill et al., (1985) Biotechnology, 3: 643-646].

Traditionally, attempts to reduce the production cycle length have focused on alteration of environmental factors, such as water temperature and photoperiod, and on selective breeding. However, such efforts are extremely costly, may require several generations to obtain a satisfactory result, and may

adversely affect the genetic diversity of the aquacultured populations. Thus, such methods have proved impractical on a large scale. Enhancement of feeding efficiency has largely been attempted by the scientific formulation of feed products, but this approach has met with limited success. More effective alternatives for reducing the length of the production cycle and increasing the efficiency of feed conversion are needed.

In 1948, Pickford and Thompson [J. Exp. Zool., 109: 367-383] reported that mammalian growth hormone administration accelerated fish growth rates. Subsequent studies have demonstrated the efficacy of mammalian growth hormones to accelerate both fish growth rates and to increase food conversion efficiency [Higgs et al., (1975) Gen. and Comp. Endocrin., 27: 240-253; Higgs et al., (1976) J. Fish Res. Board, 33: 1585-1603; Higgs et al., (1977) Can. J. Zool., 55: 1048-1056]. Mammalian prolactin has also been shown to accelerate growth in fish [Clarke et al., (1977) Gen. and Comp. Endocrin., vol 33: 174-178]. However, these results were obtained using growth hormone of the growth hormone-prolactin family obtained from natural sources. As previously mentioned, recombinant DNA technology now enables the large-scale production of recombinant growth hormones (somatotropins) from a variety of sources, including human growth hormone (hGH), bovine somatotropin (bST), porcine somatotropin (pST), chicken growth hormone (cGH), and salmon growth hormone (sGH), although an effective and economically feasible method of administration for any growth hormone to large numbers of aquacultured teleosts is still lacking.

Previous methods of administration capable of promoting sustained accelerated growth rates and

increased food conversion in fish have included intraperitoneal (ip) injection [Clarke et al., supra; Higgs et al., supra; Down et al., (1989) Can. J. Fish. Aquat. Sci., 46: 178-181] and intramuscular (im) injection [Higgs et al., (1977) supra; Down et al., (1988) Fish Phys. and Biochem., 5, no. 2: 49-57]. Growth hormone administration by injection requires the frequent handling and anesthetizing of each fish. These are labor intensive procedures that physiologically stress the fish (often resulting in high mortality rates) and are disadvantageous for use on a wide scale. Additional methods of growth hormone administration include immersion in a growth hormone-containing solution [Schulte et al., (1989) Aquaculture, 76: 145-156], implantation of a mini-osmotic pump [Down et al., (1988) Aquaculture, 68: 141-155: Down et al., (1989) J. World Aquaculture, 20: 181-187], and implantation of a hormone-containing cholesterol pellet [Higgs et al., (1976) supra; Down et al., (1988) supra]. Currently, these methods are also uneconomical. Furthermore, such methods are incapable of enabling controlled hormone delivery over the time periods necessary to significantly decrease the length of the production cycle. Presently, to achieve sustained periods of accelerated growth rates in fish, growth hormone administrations must be performed frequently and thus are impractical for use on a commercial scale for the reasons stated previously.

There presently exists a need for a means of administering growth hormones to fish in a less labor intensive manner which does not cause significant physiological stress while simultaneously producing significant periods of accelerated growth rates. Development of a controlled release growth hormone dosage form capable of producing an accelerated growth

rate over an extended time period would fulfill this goal.

Controlled release dosage forms are those formulations which enable the release of an active ingredient over a prolonged time period (for example, greater than four weeks) in a controlled and predictable manner either by degradation, disintegration, or dissolution of the dosage form, or by diffusion of the active ingredient across the membrane or matrix encapsulating the formulation. The scientific literature teaches that proteins can diffuse through hydrated polymers (hydrogels), and that the rate of protein diffusion is in part determined by the degree of polymer hydration [Pitt, C., (1990) Int. J. Pharmaceutics, 59: 173-96].

Hydrogels are three dimensional polymer networks that swell but are insoluble in an aqueous environment. A number of hydrogels, including cross-linked polymethylacrylates and polyacrylates, and cross-linked polyvinyl alcohol (PVA), have been used as controlled release matrices. Cross-linked hydrogels are typically produced by the polymerization of hydrophilic monomers in the presence of a cross-linking agent. The volume of a swollen cross-linked hydrogel is determined by the equilibrium reached between the elasticity of the elongated, cross-linked polymer chains and the force of the incoming water. The permeability of such matrices is dependent in part on the amount of cross-linking agent used in the polymerization reaction. For details, see Chemistry and Technology of Water-soluble Polymers, (1983) Editor: Finch, pp. 71-80.

SUMMARY OF THE INVENTION

It has now been discovered that non-covalently cross linked PVA compositions absorb sufficient water to become semi-permeable to growth hormones and these PVA compositions can be used in growth hormone-containing controlled release dosage forms suitable for administration to numerous non-human animal species, enabling extended periods of accelerated growth rates. Also in accordance with this invention are methods of delivering a continuous low level of growth hormone to the non-human animal being treated.

Specifically, these growth hormone-containing controlled release dosage forms are particularly well suited for implantation in aquacultured teleosts, as illustrated herein. A single administration of such a dosage form to an aquacultured teleost results in fish exhibiting the effects of growth hormone treatment over a prolonged period, substantially reduces the labor intensive nature of current administration methods, and lessens the physiological stress placed on the fish by the repeated growth hormone administrations required to obtain statistically significant growth acceleration in previous methods.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 graphically represents the release of recombinant porcine somatotropin (rpST) from a controlled release dosage form produced in accordance with this invention into a simulated physiological solution over a 12-week period. In addition, this figure represents the dependence of the growth hormone release rate on the thickness of the PVA coating.

Figure 2 graphically represents the daily water temperature measured in the communal aquarium used throughout the course of the experiment.

Figure 3 graphically represents the percent increase in body weight of experimental coho salmon implanted with controlled release rpST forms having PVA coatings of various thicknesses.

Figure 4 graphically represents the mean group weights of experimental coho salmon implanted with a PVA-coated controlled release rpST dosage form.

Figure 5 graphically represents the weight specific growth rates (%/day) of coho salmon implanted with a PVA-coated controlled release rpST dosage form.

Figure 6 graphically represents the length specific growth rates (%/day) of coho salmon implanted with a PVA-coated controlled release rpST dosage form.

Numerous aspects and advantages of the invention will be apparent to those skilled in the art upon consideration of the following detailed description which provides illumination of the practice of the invention in its preferred embodiments.

DETAILED DESCRIPTION

GROWTH HORMONE

Various growth hormones, including bST [Higgs et al., supra; Down et al., Can. J. Fish. Aquat. Sci., (1989) 46: 178-83], cGH [Gill et al. supra; Down et al., Fish Phys. & Biochem., (1988) 5: 49-57], hGH

[Pickford et al., supra], ovine prolactin [Clarke et al., supra], and sGH [Sekine et al., supra] have been shown to promote growth in various fish species, such as salmon [Higgs et al., supra; Down et al., Aquaculture, (1988) 68: 141-155], trout [Schulte et al., Aquaculture, (1989) 76: 145-156; Le Bail et al., J. of Exp. Zoo., (1989) 251:101-107], and eels [Degani et al., Can. J. Fish. Aquat. Sci., (1985) 42: 185-189]. Recombinant DNA technology has enabled the production of growth hormones in quantities, and quality, sufficient for commercial application. Thus, in a preferred embodiment of the present invention, the growth hormone used is derived from a recombinant source. Especially preferred are recombinantly derived porcine and bovine somatotropin, rpST and rbST, respectively.

In vivo, growth hormones promote the construction of protein from amino acids, an initial fall in plasma glucose upon administration, a gradual rise in plasma glucose subsequent to the initial fall, and a breakdown of fats into fatty acids, respectively referred to as growth promotion, insulin-sparing, diabetogenic, and lipolytic effects. Discrete portions of the growth hormone protein molecule are responsible for one or another of these effects. A naturally occurring variant of hGH, having several sequential amino acids deleted, has been previously isolated and demonstrated to lack lipolytic and insulin-sparing activities [Lewis et al., J. Biol. Chem., (1978) 253: 2679-2687: Lewis et al., Endocr. Res. Commun., (1981) 8: 155-164]. Therefore, the present invention encompasses growth hormone analogs of the growth hormone-prolactin family having certain domains deleted from the protein, either by modification of the gene encoding the amino acids for the hormone, differential

processing of mRNA specifying said protein, chemical or proteolytic cleavage of the mature protein molecule, or by any other method, but still leaving said analog endowed with some, or perhaps improved, growth promoting activity. Such growth rate accelerating analogs of growth hormone suitable for administration to various non-human animal species have been produced for pST [European Patent Appl. No. 0 282 319] and bST [Down et al., (1989) supra]. In addition, the present invention envisions the use of other recombinant growth hormone analogs possessing greater stability or higher activity resulting from the insertional or substitutional modification of the polypeptide's primary structure.

Biological activity is an important aspect of any growth hormone molecule of the growth hormone-prolactin family, be it natural, recombinant or modified. Because the present invention makes possible prolonged growth rate acceleration in non-human animals, including fish, the growth hormone used must be of sufficiently high activity such that sufficient quantities of growth hormone can be incorporated into a controlled release dosage form capable of being implanted into non-human animals, especially relatively small animals, such as immature fish.

It is estimated that the normal range of plasma growth hormone in fish ranges from 0.1 - 100 ng/ml, and is usually 1 - 10 ng/ml. Maintenance of plasma growth hormone concentrations at 100 - 200 ng/ml produce marked increases in salmon growth responses [Down et al., (1988) supra]. The controlled release formulation in accordance with the present invention enables maintenance of growth hormone at concentrations sufficient to produce extended periods of accelerated growth. In general, the present invention provides for

maintenance of plasma growth hormone levels above 10 ng/ml.

GROWTH HORMONE-CONTAINING PELLET

Promotion of accelerated growth rates in non-human animal species over an extended time period requires that the growth hormone promoting such growth be available in sufficient quantities to maintain a level of biological activity necessary to promote an accelerated growth rate for the intended period. Excipients are biologically inert molecules used throughout the pharmaceutical industry in formulations as bulking agents, stabilizing ingredients, etc. Examples of excipients suitable for use in accordance with this invention include chitosan, sucrose, serum albumin, mannitol, dextran, and water soluble PVA. These excipients can be combined with the described growth hormone to provide increased stability or to facilitate preparation of the controlled release dosage form. Chitosan has been found to be effective for use in the formulation of growth hormone into controlled release dosage forms. A mixture of chitosan and growth hormone can contain a range from about 1 to about 99 parts by weight of chitosan and from about 99 to about 1 part by weight of growth hormone. Particularly preferred are compositions of about 55 to 65 parts by weight of growth hormone and about 45 to 35 parts by weight of chitosan (based on 100 parts by weight of the two compounds when combined). Such a mixture is effective for producing a composition that can be compressed or otherwise formulated into a suitable vehicle for growth hormone delivery to non-human animals, particularly fish. Preferably a pellet containing chitosan and growth hormone is manufactured

by compression of the chitosan/growth hormone composition. Such a manufactured pellet, whose size and shape is dependent upon the amount of material compressed, the type of press used and the compression force employed, can then be coated with an appropriate polymer to render it suitable for administration to fish as a controlled release dosage form. In administering the disclosed dosage form to aquacultured teleosts, the size of the manufactured growth hormone/chitosan-containing pellets should be appropriate for the size of the fish to which the formulation is to be administered, as those skilled in the art will appreciate.

For the purposes of the present invention, the term growth promoting activity shall be construed to mean either of the following: (1) growth promoting hormones composed of amino acids, also referred to as proteinaceous growth hormones; and (2) growth promoting hormones not composed of amino acids, also called non-proteinaceous growth hormones.

Combinations of growth promoting activities can also be used in the practice of this invention. For instance, mixtures of bST, a proteinaceous growth hormone, with other non-proteinaceous growth-promoting hormones, such as 17a-methyltestosterone and L-thyroxine, are also known to promote accelerated growth rates in fish in a synergistic manner. [Higgs et al., Can. J. Zool., (1977) 55:1048-1056: Degani et al., supra]. Thus, one aspect of the present invention envisions a combination of various proteinaceous and non-proteinaceous growth hormones encapsulated by a polymer capable of controlling the diffusion rate of the growth promoting activities so as to produce a dosage form capable of sustaining accelerated growth

rates in a variety of non-human animal species over a prolonged time period.

CONTROLLED RELEASE DEVICE

PVA may be prepared by the hydrolysis of polyesters, e.g., polyvinyl acetate (PVAc), polyvinyl trifluoroacetate, or polyvinyl t-butyl ether. Incomplete hydrolysis results in residual ester or ether groups in some positions in the polymer. Thus, preparations of PVA most often are not homopolymers, but instead are carbon-based polymer backbones substituted with varying amounts of ester or ether side groups, which can effect the polymer's tacticity, crystallinity, and solubility in various solvents. Unlike covalently cross-linked hydrogels, non-covalently cross-linked PVA preparations with certain molecular weights, tacticities, crystallinities, and degrees of hydrolysis are insoluble in water at 37°C, but may be dissolved in aqueous-organic mixtures.

For example, non-covalently cross-linked PVA derived from polyvinyl acetate that is 99% hydrolyzed is not soluble in water, but is soluble in solutions containing up to 25% aqueous ethanol. Within the scope of the present invention, the effective range of PVA hydrolysis ranges from about 95% to 100%, with greater than 99% hydrolysis being the most preferred. A PVA preparation with this degree of hydrolysis is soluble in aqueous solutions containing up to 25% ethanol. In particular, a 25% aqueous ethanol solution is preferred. Such aqueous-organic solutions of non-covalently cross-linked PVA compositions may then be cast as films, extruded as tubing, or sprayed as coatings. Decreased degrees of hydrolysis lead to increased water solubility, reducing the suitability of

such compositions for use as semipermeable membranes in aqueous environments.

To serve effectively as a means of controlling the diffusion rate in a controlled release formulation in accordance with this invention, it is best to apply the PVA solution as a coating to the external surface of the pellet containing the growth hormone. Such application can be accomplished by the use of conventional techniques like dipping, pan coating, or, more preferably, by spray coating the pellet. Such a process insures a coating of relatively uniform thickness over the entire pellet surface, as well as a coating relatively free from defects.

The diffusion rate of the growth hormone is inversely proportional to the thickness of the PVA coating applied. Coating thickness is a function of the length of time a growth hormone-containing pellet is exposed to a spray composed of the PVA solution, whereby at a constant rate of spraying, the longer the spray period and/or the more concentrated the solution, the thicker the coating. Thus, coatings of various thicknesses can be applied utilizing spray periods of differing lengths and/or solutions containing different concentrations of PVA. Therefore, the time period over which the controlled release formulation will maintain an accelerated growth rate in fish or any other animal species is dependent upon factors such as the thickness of the PVA coating, the surface area and size of the pellet, and the amount of growth enhancing activity contained in the pellet. It is possible to achieve spray-applied coatings with PVA thicknesses of less than 1μm to more than several 100μm. However, PVA coating thicknesses ranging particularly from 25 - 100μm, when applied to growth hormone-containing pellets as described in this invention, have been found

to enable significant periods of accelerated growth rates in non-human animals, particularly fish.

The choice of the thickness of the coating applied, the size of the pellet, the amount of growth promoting activity incorporated into the the pellet, etc. will depend upon the type and size of the non-human animal to be treated, the time period over which an accelerated growth rate is desired, and other factors of which those skilled in the art are aware.

In addition, PVA may be blended with one or more other water swellable polymers prior to being applied to the formulation containing growth hormone. As will be familiar to those skilled in the art, blending of two or more polymers may be used to control the permeability of applied coatings and matrices [Cha et al., (1988) J. Controlled Release, 7: 69-78; Pitt et al., (1987) Proc. Int. Symp. Controlled Release of Bioactive Materials, 14: 75]. Such mixtures may be used to optimize the rate of release of the growth promoting activity. Other polymers suitable for blending with PVA include hydroxypropyl cellulose, hydroxypropyl methylcellulose, polyvinyl pyrrolidone, poly(glycolic acid-co-lactic acid), non-crosslinked poly(2-hydroxyethyl methacrylate), and chitosan. In yet another embodiment, the PVA-coated pellet may have a second coating applied. Again, this latter coating could serve to optimize the rate of release of the growth promoting activity from the pellet. This latter coating could also serve to prevent diffusion of rpST from the pellet until some future time, when the outer coating will have been degraded or otherwise will have lost its ability to prevent diffusion of growth promoting activity from the controlled release dosage form.

ADMINISTRATION

Because the purpose of this invention is to produce accelerated rates of growth in non-human animal species, particularly to reduce the length of the production cycle in teleost aquaculture, the dosage form of this invention should be administered early in the life cycle, although later administration, prior to fish reaching a mature size, is possible. For salmon, controlled release dosage forms can be delivered to fish as small as two grams.

The controlled release dosage form of this invention can be implanted either intraperitoneally, subcutaneously, or intramuscularly. Implantation of the dosage form can either be performed surgically or by injection. When administered to fish, prior to implantation, the teleost may be anesthetized by methods known by those skilled in the art. If risk of infection exists post-implantation, antibiotics to prevent such infection may be employed.

Likely candidates for implantation of the controlled release dosage forms described in the present invention include any commercially exploited non-human animal species, such as cattle, fowl, goats, pigs, sheep, in addition to a number of fish species (both salt and fresh water) which can be aquacultured, particularly *Anguilla* species (all cultivated eels), all aquacultured *Cyprinid* species (carp), catfish, cichlids, mullet, sea breams, sea bass, grouper, milkfish, cod, sturgeon, halibut, turbots, ornamental fish species, and several salmonid species, including *Salmo salar* (Atlantic salmon), *Oncorhynchus tshawytscha* (chinook), *O. kisutch* (coho), *O. keta* (chum), *O. nerka* (sockeye), *O. gorbuscha* (pink) *O. masou* (masu), and *O. mykiss*.

Use of the dosage forms of this invention enable the controlled release of growth hormone to fish in biologically effective quantities (that which results in periods of accelerated growth rates) over periods of four weeks or more, and typically longer than 20 weeks. In larger non-human animals, especially those that are warm blooded, effective periods of sustained accelerated growth rates are typically two weeks.

The following examples are offered to more fully illustrate the present invention, but are not meant to limit the scope thereof.

EXAMPLE 1

Preparation of PVA-encapsulated pST implant device

Eight grams of lyophilized recombinant pST and five grams of chitosan were micronized separately at 23°C to a particle size of less than 125µm. Particles having a diameter greater than 125µm were removed by sieving. The chitosan and rpST were then mixed in a ratio of about 60 parts rpST to about 40 parts chitosan to achieve a uniform composition.

Pellets were made by compressing the rpST/chitosan mixture in a tabletting machine (Manesy Machines, Ltd.) using a 1/8" diameter concave punch and die (Thomas Engineering, Inc.) at 23°C, yielding spherical pellets of approximately 23mg in weight. In addition, placebo pellets containing no growth hormone were produced by compressing a microcrystalline cellulose composition containing 2.5% magnesium stearate. Production of larger pellets, suitable for administration to larger non-human animal species, may be accomplished by compressing a larger quantity of the rpST/chitosan mixture in a larger compression device.

The resulting pellets, both the placebo and those containing rpST, were spray coated in a fluidized bed coater (Coating Place, Inc.) at 60°C with a 2.5% w/v solution of PVA (99.7% mole hydrolysed, MW 78,000) solubilized in 25% aqueous ethanol. The duration of the spraying procedure controlled the thickness of the PVA coating applied. Four thicknesses were produced. Scanning electron microscopy determined the thicknesses to be 25μm, 50μm, 70μm, and 75μm, designated as lots A, B, C, and D, respectively. Scanning electron microscopy showed no defects in the PVA coatings.

EXAMPLE 2

In vitro release of rpST from the implant device

To ascertain whether the PVA encapsulated rpST-containing pellets resulted in the controlled release of rpST from the pellets over time, studies simulating physiological conditions were conducted as follows.

Pellets from each of the four lots (A-D) with PVA-coating thicknesses of 25μm, 50μm, 70μm, and 75μm, respectively, were immersed in separate reservoirs of phosphate buffered saline, pH 7.4 (PBS), at 4°C. Each reservoir was gently agitated. Aliquots from each reservoir were withdrawn on a weekly basis to ascertain the rpST released by measurement of the UV absorbance at 280nm. The PBS in each reservoir was changed whenever the A_{280} reached 0.10 absorbance units or greater. The rates of release of rpST from pellets with various coating thicknesses are shown in Figure 1. The release rate of rpST from the controlled release dosage forms is seen to be inversely proportional to the PVA coating thickness.

The identity and purity of the released rpST was confirmed by HPLC. Comparison with a standard sample of rpST demonstrated that the purity remained unchanged throughout the course of the experiment.

Release rate measurements were discontinued after 124 days, at which time the pellets were examined to determine the amount of protein remaining in the pellets. rpST was extracted from the pellets by crushing them in fresh PBS to dissolve the contents. HPLC and UV absorbance measurements were used to determine the purity and quantity of residual protein in the pellets, respectively (see TABLE 1).

TABLE 1

SAMPLE	PVA COATING THICKNESS	rpST RELEASED mg	rpST RELEASED % OF TOTAL	% OF TOTAL	RESIDUAL pST % OF MONOMERIC PROTEIN
A	25 µm	11.4 ± 0.8	100.2 ± 7.6	8.6 ± 2.1	74.5 ± 3.7
B	50 µm	9.8 ± 0.1	85.6 ± 0.7	26.4 ± 8.6	79.8 ± 1.1
C	70 µm	3.0 ± 0.8	26.2 ± 7.2	77.3 ± 2.9	83.4 ± 1.3
D	75 µm	2.4 ± 0.0	21.3 ± 0.3	80.5 ± 0.3	83.4 ± 0

EXAMPLE 3

In vivo administration

One hundred size-selected coho salmon (*O. kisutch*, 67.0 ± 4.7 g wet weight, 17.5 ± 0.48mm fork length) were chosen from a group of 2,100 individuals obtained from the Capilano Salmon Hatchery (North Vancouver, B.C.). The selected fish were randomly assigned to one of five experimental groups. All groups were maintained in a communal outdoor 7,000 l flow-through aquarium (3m diameter, 1m depth) under ambient photoperiod, salinity, and temperature (Figure 2). Throughout the experiment fish were fed to satiation three times daily on a commercial semi-moist diet (Oregon Moist Pellets, Moore Clark, Laconner, WA). During the experiment, all fish were treated identically with respect to handling, anesthetization, and transport.

Implantation of the controlled release dosage forms and individual identification was accomplished by first slightly anesthetizing each fish with 2-phenoxyethanol (0.04%, Syndel Labs, Vancouver, B.C.) and then making a small incision slightly to one side of the midventral line and positioned approximately 1 cm anterior to the insertions of the pelvic fins. A single controlled release dosage form and a passive integrated transponder (Identification Devices, Inc., Boulder, CO) were then placed into the peritoneal cavity of each salmon. Incisions were closed with 2-0 braided silk (Ethicon Sutures Ltd., Peterborough, Ont.) using a single interrupted horizontal mattress suture. Fish from one group received placebo pellets. Each of the other groups were implanted with growth hormone-containing pellets with PVA coatings of a specific thickness, either 25μm, 50μm, 70μm, or 75μm.

After implantation of the controlled release dosage forms, each fish was weighed to the nearest 0 1g and its fork length measured to the nearest millimeter. Each fish was then returned to the communal aquarium. Once a week for 6 weeks, and then once every other week, each fish was again lightly anesthetized with 0.04% 2-phenoxyethanol. They were then weighed to the nearest 0.1g and fork lengths were measured to the nearest millimeter. Data was always collected between 08:00h - 10:00h. In addition, fish were fasted on sampling days.

At the termination of the data collection period (20 weeks), fish performance was expressed by weight gain, as both percentage weight gain (Figure 3) and in absolute units (Figure 4) over the course of the study, weight-specific growth rates of the various experimental groups (Figure 5), as well as by length-specific growth rates (Figure 6). These results indicate that pellets having the thinnest PVA coating (25μm) promote the highest growth rates. Performances of the rpST treated groups were examined statistically against the placebo-implanted control group by one way analysis of variants followed by Duncan's multiple range test of significance.

* * *

While the present invention has been described in terms of preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art in light of the above description. Therefore, it is intended that the appended claims cover all such variations which come within the scope of the invention as claimed.

WHAT IS CLAIMED IS:

1. A controlled release dosage form suitable for administration to a non-human animal comprising:

A) a growth promoting substance in an amount sufficient to accelerate the growth rate of the animal; and

B) a coating for the growth promoting substance comprising a water swellable non-covalently cross-linked polyvinyl alcohol through which the growth promoting substance is capable of diffusing.

2. A controlled release dosage form according to Claim 1 wherein the non-human animal is selected from the group consisting of cattle, chickens, ducks, fish, geese, goats, pigs, sheep, and turkeys.

3. A controlled release dosage form according to Claim 2 wherein the non-human animal is a fish.

4. A controlled release dosage form according to Claim 1 wherein the growth promoting substance is a hormone of the growth hormone-prolactin family.

5. A controlled release dosage form according to Claim 1 wherein the growth promoting substance is selected from the group consisting of avian growth hormone, mammalian growth hormone, and teleost growth hormone.

6. A controlled release dosage form according to Claim 5 wherein the growth promoting substance is mammalian growth hormone.

7. A controlled release dosage form according to Claim 6 wherein the mammalian growth hormone is selected from the group consisting of porcine somatotropin and bovine somatotropin.

8. A controlled release dosage form according to Claim 7 wherein the mammalian growth hormone is derived from a recombinant source.

9. A controlled release dosage form according to Claim 7 wherein the mammalian growth hormone is a mammalian growth hormone analog derived from a recombinant source.

10. A controlled release dosage form according to Claim 8 wherein the mammalian growth hormone is recombinant porcine somatotropin.

11. A controlled release dosage form according to Claim 1 wherein the growth promoting activity is comprised of a combination of a proteinaceous growth hormone and a non-proteinaceous growth hormone.

12. A controlled release dosage form according to Claim 5 wherein the growth promoting substance is a teleost growth hormone.

13. A controlled release dosage form according to Claim 12 wherein the teleost growth hormone is derived from a recombinant source.

14. A controlled release dosage form according to Claim 12 wherein the teleost growth hormone is a teleost growth hormone analog derived from a recombinant source.

15. A controlled release dosage form according to Claim 13 wherein the teleost growth hormone is recombinant salmon growth hormone.

16. A controlled release dosage form according to Claim 1 wherein the polyvinyl alcohol is characterized by a degree of hydrolysis of no less than about 95%.

17. A controlled release dosage form according to Claim 16 wherein the polyvinyl alcohol is characterized by a degree of hydrolysis from about 95% to 100%.

18. A controlled release dosage form according to Claim 1 wherein the rate of diffusion of the growth hormone is inversely proportional to the coating thickness.

19. A controlled release dosage form according to Claim 1 wherein the coating comprises an admixture of polyvinyl alcohol with one or more other water-swellable polymers.

20. A controlled release dosage form according to Claim 1 which includes one or more excipients selected from the group consisting of chitosan, sucrose, serum albumin, mannitol, dextran, and water-soluble polyvinyl alcohol.

21. A controlled release dosage form according to Claim 20 wherein the excipient is chitosan.

22. A controlled release dosage form according to Claim 1 wherein the coating has been applied by a process selected from the group consisting of spray coating, pan coating, and dip coating.

23. A controlled release dosage form according to Claim 22 wherein the coating has been applied by spray coating.

24. A controlled release dosage form according to Claim 23 wherein the spray applied coating has a thickness from less than 1μm to more than 100μm.

25. A controlled release dosage form according to Claim 24 wherein the coating thickness ranges from about 25μm to 100μm.

26. A controlled release dosage form according to Claim 25 wherein the coating thickness is about 25μm.

27. A method of accelerating the growth rate of a non-human animal by administering to the animal a controlled release dosage form comprising:

A) a growth promoting substance in an amount sufficient to accelerate the growth rate of the animal; and

B) a coating for the growth promoting substance comprising a water swellable non-covalently cross-linked polyvinyl alcohol through which the growth promoting substance is capable of diffusing.

28. A method according to Claim 27 wherein the non-human animal is selected from the group consisting of cattle, chickens, ducks, fish, geese, goats, pigs, sheep, and turkeys.

29. A method according to Claim 28 wherein the non-human animal is a fish.

30. A method according to Claim 27 wherein the growth promoting substance is a hormone of the growth hormone-prolactin family.

31. A method according to Claim 27 wherein the growth promoting substance is released over a period of four or more weeks.

32. A method according to Claim 31 wherein the growth promoting substance is released in fish over a period or 4 or more weeks.

33. A method according to Claim 32 which is applied to fish selected from the group consisting of basses, carps, catfishes, cichlids, chars, cods, eels, groupers, halibuts, milkfish, mullets, ornamental fish species, salmonids, sea breams, sturgeons, trouts, and turbots.

34. A method according to Claim 33 wherein the fish is a salmonid selected from the genera consisting of Oncorhynchus, Salmo or Salvelinus.

35. A method according to Claim 27 wherein the growth promoting substance is selected from the group consisting of teleost growth hormone, mammalian growth hormone, and avian growth hormone.

36. A method according to Claim 35 wherein the growth promoting substance is mammalian growth hormone.

37. A method according to Claim 36 wherein the mammalian growth hormone is selected from the group consisting of porcine somatotropin and bovine somatotropin.

38. A controlled release dosage form according to Claim 37 wherein the mammalian growth hormone is a mammalian growth hormone analog derived from a recombinant source.

39. A method according to Claim 37 wherein the growth hormone is derived from a recombinant source.

40. A method according to Claim 39 wherein the growth hormone is recombinant porcine somatotropin.

41. A controlled release dosage form according to Claim 27 wherein the polyvinyl alcohol is characterized by a degree of hydrolysis of no less than about 95%.

42. A method according to Claim 41 wherein the polyvinyl alcohol is characterized by a degree of hydrolysis from about 95% to 100%.

43. A method according to Claim 27 wherein the polyvinyl alcohol coating thickness is inversely proportional to the rate of diffusion of the growth hormone.

44. A method according to Claim 27 wherein the coating comprises an admixture of polyvinyl alcohol with one or more other water swellable polymers.

45. A method according to Claim 27 which includes one or more excipients selected from the group consisting of chitosan, sucrose, serum albumin, mannitol, dextran, and water soluble polyvinyl alcohol.

46. A method according to Claim 45 wherein the excipient is chitosan.

47. A method according to Claim 27 wherein the coating has been applied by a process selected from the group consisting of spray coating, pan coating, and dip coating.

48. A method according to Claim 47 wherein the coating has been applied by spray coating.

49. A method according to Claim 48 wherein the spray applied coating has a thickness from less than 1μm to more than 100μm.

50. A method according to Claim 49 wherein the coating thickness ranges from 25μm to 100μm.

51. A method according to Claim 50 wherein the coating thickness is about 25μm.

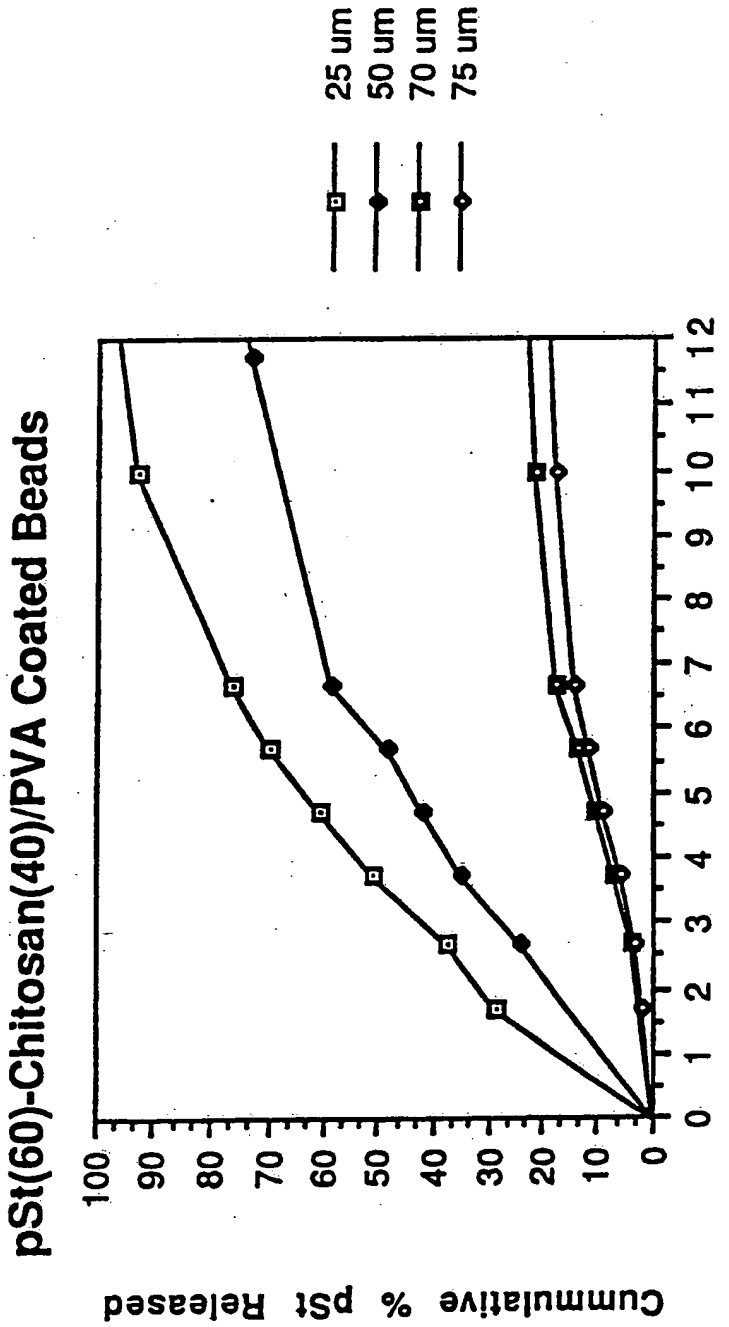
25 um
50 um
70 um
75 um
pSt(60)-Chitosan(40)/PVA Coated Beads
Cummulative % pSt Released
0
10
20
30
40
50
60
70
80
90
100
0 1 2 3 4 5 6 7 8 9 10 11 12
Weeks

FIG. I

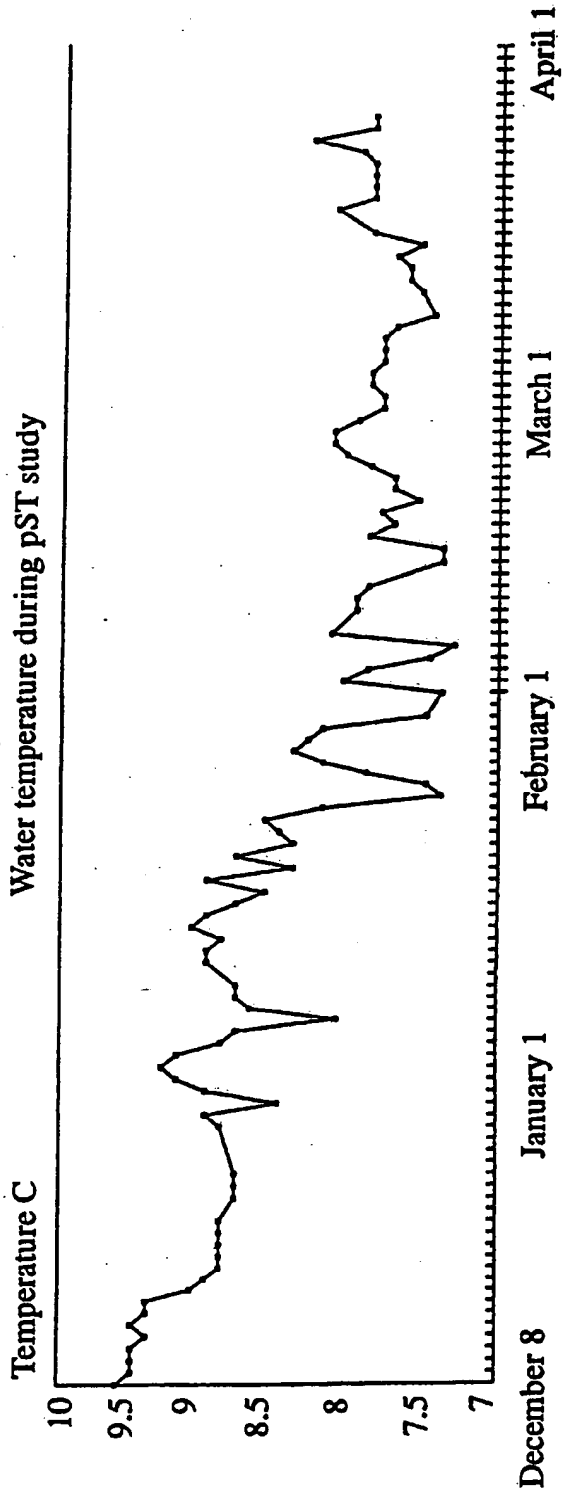
Water temperature during pST study
Temperature C
10
9.5
9
8.5
8
7.5
7
December 8
January 1
February 1
March 1
April 1
Month (1989/90)

FIG. 2

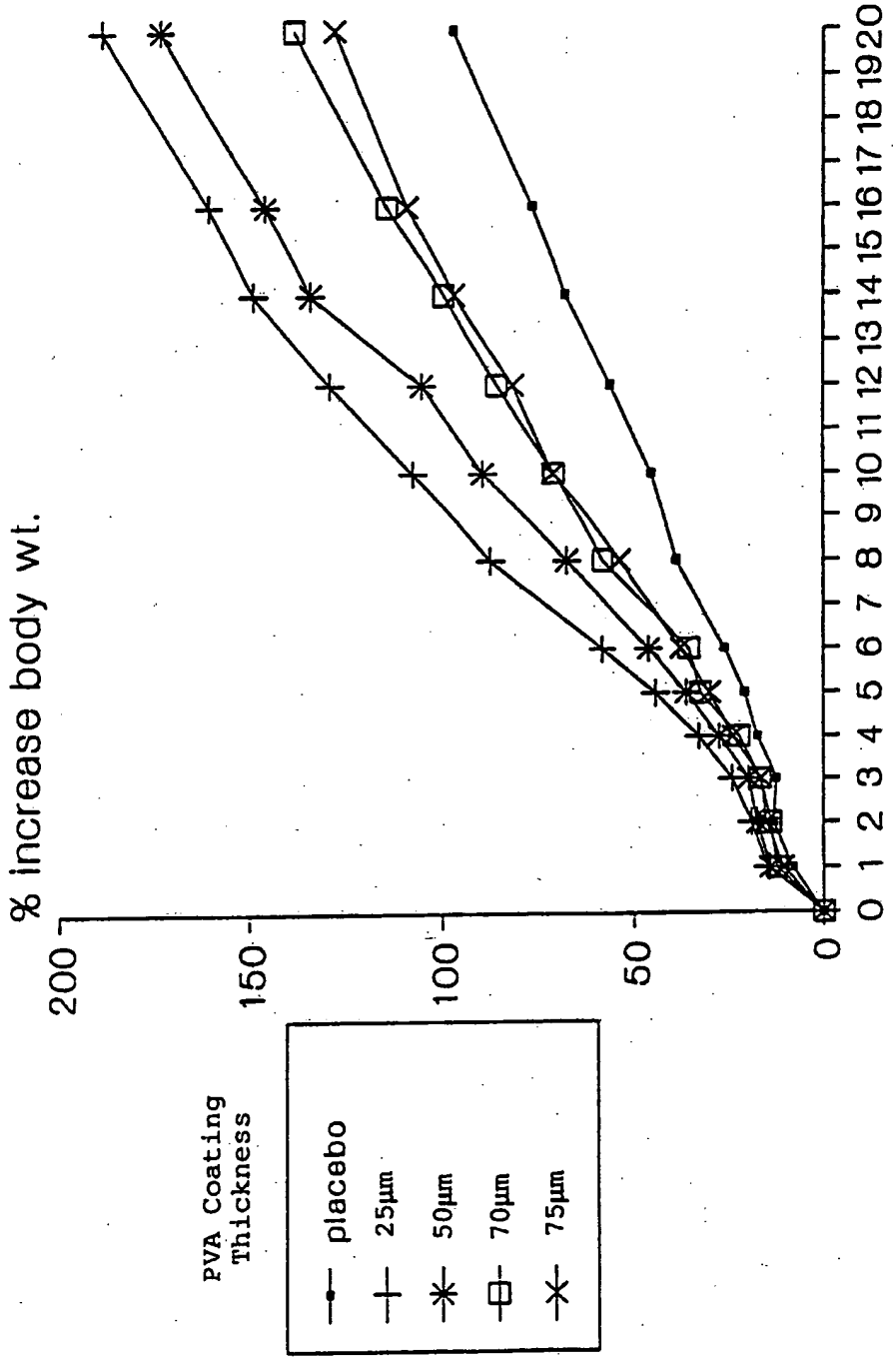
% increase body wt.
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
time (wk)
PVA Coating Thickness
placebo
25µm
50µm
70µm
75µm

FIG. 3

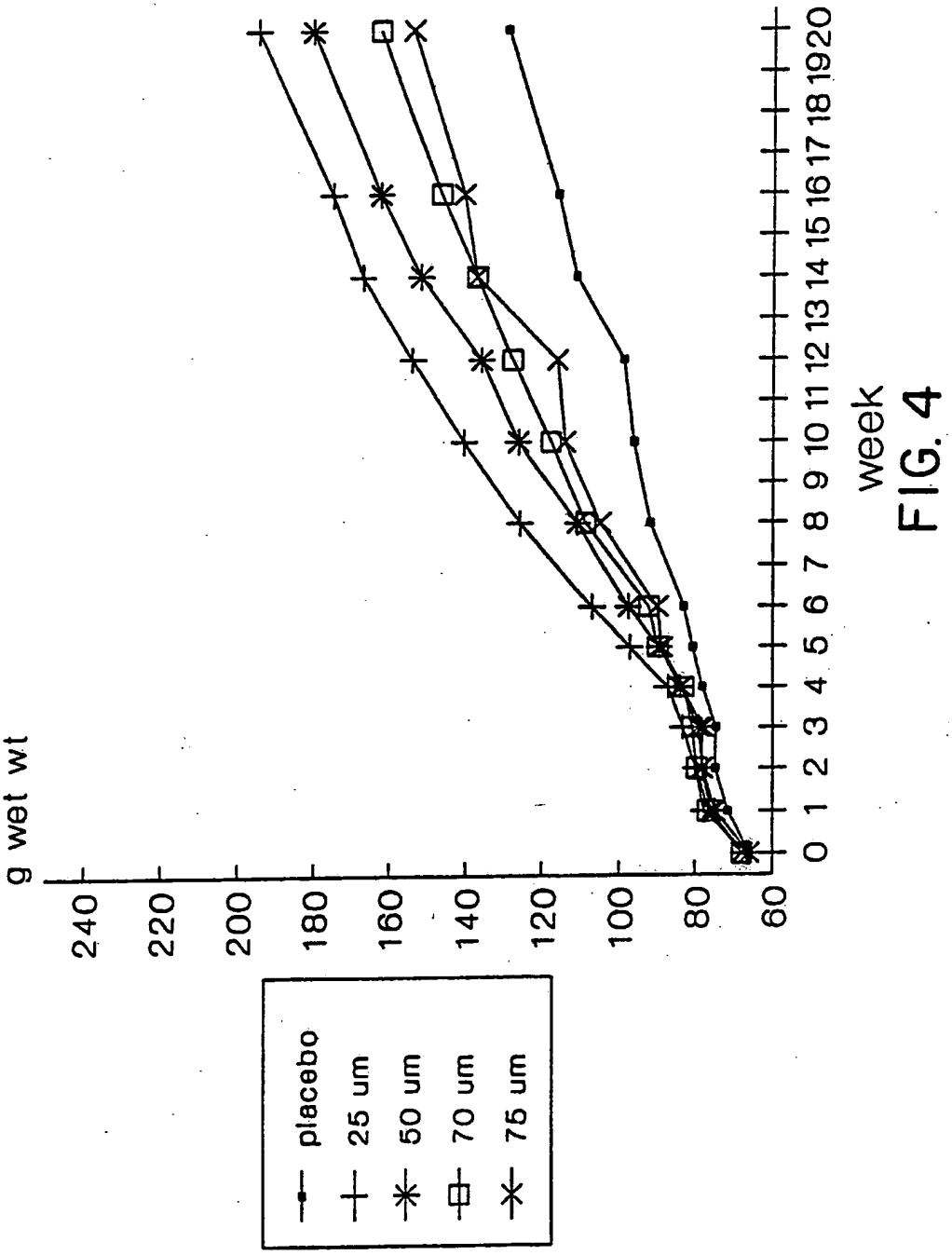
g wet wt
240
220
200
180
160
140
120
100
80
60
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
week
placebo
25 um
50 um
70 um
75 um

FIG. 4

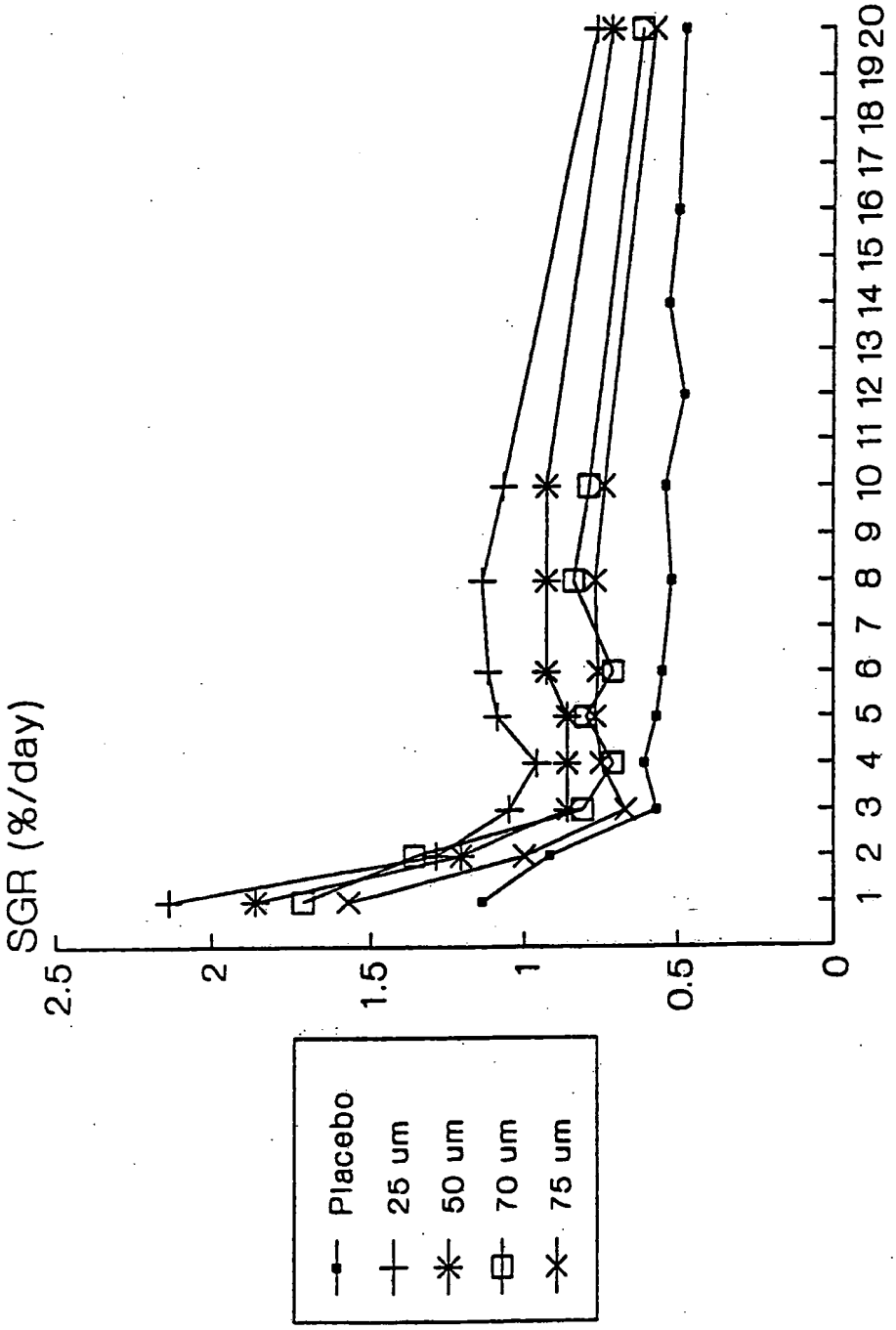
SGR (%/day)
2.5
2
1.5
1
0.5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
week
Placebo
25 um
50 um
70 um
75 um

FIG. 5

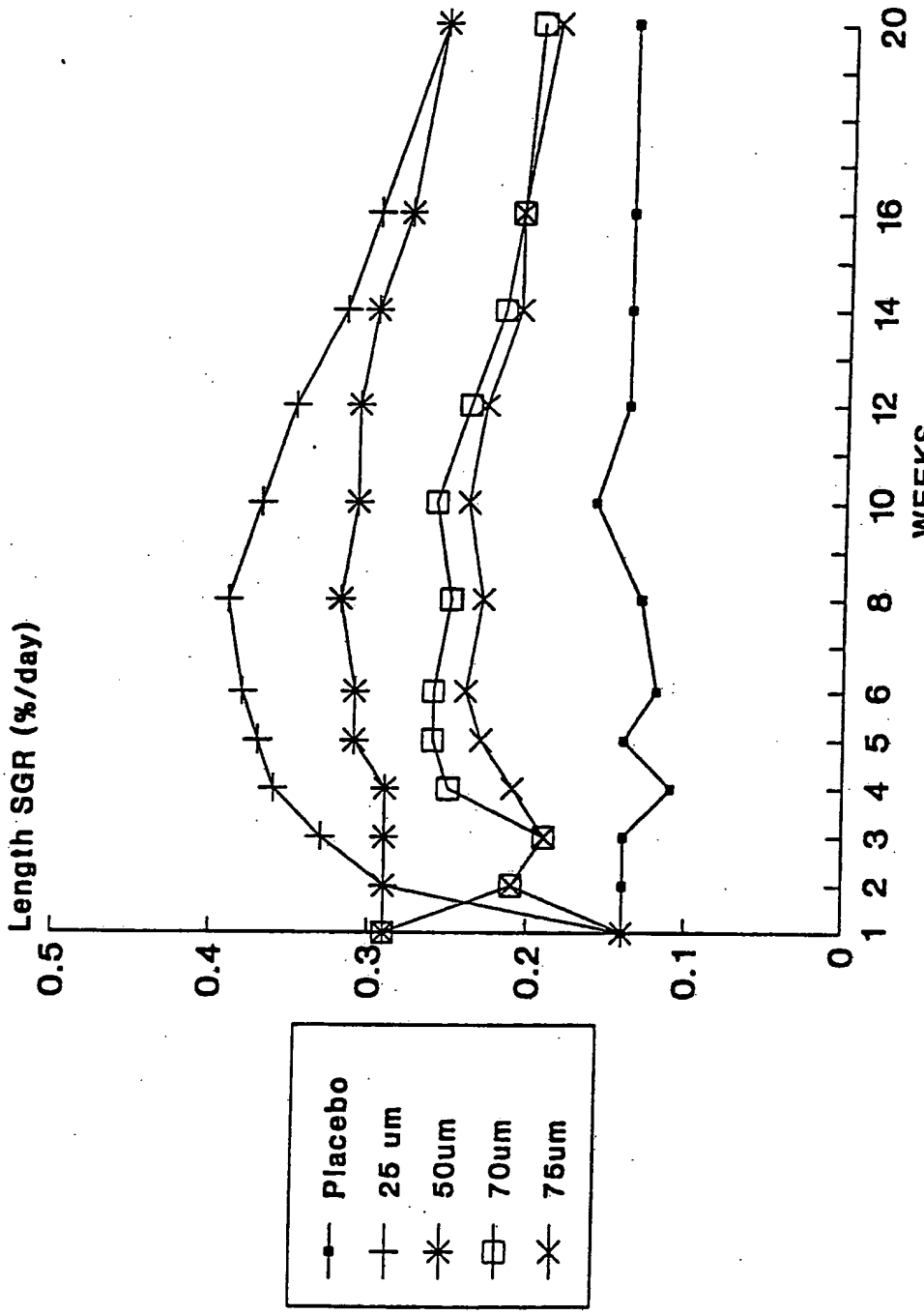
Length SGR (%/day)
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 8 10 12 14 16 20
WEEKS
Placebo
25 um
50um
70um
75um

FIG. 6

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/08129

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A61K 9/32
U.S. CL. 424/482

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	424/482

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4,849,359 (SEKINE) 18 JULY 1989 See Examples 5 and 7.	12-15
Y	US, A, 4,786,501 (JANSKI) 22 NOVEMBER 1988 See the Title; column 1, lines 45-56; column 5, line 67; column 6, line 2; column 6, lines 61-65; column 7, lines 1-8.	1-10, 16-19, 22-30, 35-45, 47-51
Y	US, A, 4,895,724 (CARDINAL) 23 JANUARY 1990 See column 3, line 1 and column 2, lines 10-18.	20 & 21
Y	US, A, 4,808,353 (NAMBU) 28 FEBRUARY 1989 See column 2, lines 58-60; column 5, lines 56-57; column 6, lines 66-68; column 7, lines 18-26 and column 7, lines 38-54.	1-3, 16, 17, 27-29, 41, 42
Y	US, A, 4,645,755 (KAWAUCHI) 24 FEBRUARY 1987 See column 5, line 14.	31-34

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
30 DECEMBER 1991	22 JAN 1992
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Edward J. Webman

Form PCT/ISA/210 (second sheet) (Rev.11-87)